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Evidence of an association between sleep and levodopa-induced dyskinesia in an animal model of Parkinson's disease

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ABSTRACT

Levodopa-induced dyskinesia (LID) represents a major challenge for clinicians treating patients affected by Parkinson's disease (PD). Although levodopa is the most effective treatment for PD, the remodeling effects induced by disease progression and the pharmacologic treatment itself cause a narrowing of the therapeutic window because of the development of LID. Although animal models of PD provide strong evidence that striatal plasticity underlies the development of dyskinetic movements, the pathogenesis of LID is not entirely understood. In recent years, slow homeostatic adjustment of intrinsic excitability occurring during sleep has been considered fundamental for network stabilization by gradually modifying plasticity thresholds. So far, how sleep affects on LID has not been investigated. Therefore, we measured synaptic downscaling across sleep episodes in a parkinsonian animal model showing dyskinetic movements similar to LID. Our electrophysiological, molecular, and behavioral results are consistent with an impaired synaptic homeostasis during sleep in animals showing dyskinesia. Accordingly, sleep deprivation causes an anticipation and worsening of LID supporting a link between sleep and the development of LID.

1. Introduction

Levodopa (LD)—induced dyskinesia (LID) is difficult to treat, negatively affects quality of life, and increases the treatment costs of Parkinson's disease (PD) patients (Dodel et al., 2001). Although LD currently represents the most effective treatment for PD patients (Poewe et al., 2010), ameliorating cardinal signs such as bradykinesia, akinesia and rigidity (Katzenschlager and Lees, 2002), these benefits are in some measure overshadowed by the emergence of LID (Jankovic and Stacy, 2007). In the early stages, PD patients usually experience an acceptable quality of life that is impaired in the advanced stages by the emergence of these involuntary movements that frequently occur at the peak of the LD effect (Berthet and Bezard, 2009; Nadjar et al., 2009). In other words, when the patients experience motor complications such as a shortening motor response and the development of dyskinesia, the delivery of LD without inducing dyskinesia becomes increasingly difficult (Jankovic and Stacy, 2007; Olanow et al., 2006).

Several attempts have been made to find "pure" anti-dyskinetic drugs, able to uncouple the anti-akinetic effect from the dyskinetic one of LD and other dopamine (DA) agonists. As a consequence, great efforts have been made to clarify LID pathogenesis, emphasizing the role of pulsative stimulation of striatal receptors by dopaminergic treatment (Olanow et al., 2006), and also changes of postsynaptic (Calabresi et al., 2010; Jenner, 2008) or presynaptic processes at the striatal level (Lunardi et al., 2009). Although no conclusive results on LID pathogenesis have been achieved, disease duration, that is, the degree of dopaminergic degeneration, along with long-term use of LD, seems to play a crucial role (Berthet and Bezard, 2009; Nadjar et al., 2009). This is perceivable in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine—induced parkinsonism in humans in whom the extended dopaminergic damage caused

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within a few days of LD treatment the development of LID undistinguishable from those in the idiopathic form (Langston and Ballard, 1984). Promising results with respect to LID pathogenesis come from animal models and PD patients demonstrating an uncontrolled long-term potentiation (LTP) of corticostriatal synapses in association with the development of LID (Calabresi et al., 2000; Picconi et al., 2003). Striatal medium spiny neurons of dyskinetic rats have an impaired reversal of the previously induced LTP, that is, "depotentiation" (Picconi et al., 2003). Furthermore, PD patients showing LID were unresponsive to the depotentiation paradigm suggesting that depotentiation is abnormal in the motor cortex of patients with LID (Huang et al., 2011). Dyskinetic movements can be interpreted as intruding motor programs in selected neuronal responses because of unrestrained LTP. Physiological control of spike-timing–dependent plasticity (STDP) such as LTP has a crucial role in network stability (Turrigiano and Nelson, 2004). Recently, an important role for sleep and in particular for electroencephalogic (EEG) slow-wave activity (SWA) has been proposed. According to the concept of synaptic homeostasis (SH) hypothesis, SWA is believed to underlie an overnight homeostatic process, by which neuronal circuit activity is not driven toward runaway excitation by LTP (Tononi and Cirelli, 2014). Moreover, SWA spectral density analysis represents a reliable measure of synaptic downscaling during nonrapid eye movement (NREM) sleep (Tononi and Cirelli, 2014; Vyazovskiy et al., 2007) and recent compelling evidence associated SWA to cortical synaptic strength and therefore measuring the reduction in SWA from early (the first 1 hour at the beginning of the light period) to late (the last 1 hour of the light period) NREM sleep has often been used as a direct measure of SH (Vyazovskiy et al., 2007).

STDP and homeostatic plasticity involve, in different ways, the trafficking of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) by different calcium dynamics. Some signaling molecules are linked to postsynaptic expression of AMPARs, and among these, the Arc protein seems to be a critical mediator of this cellular phenomenon (Béïque et al., 2011). A physiological decrease in Arc has been demonstrated during sleep (Hanlon et al., 2009), and an increased level of the Arc protein has been shown in the striatum of dyskinetic rats (Sgambato-Faure et al., 2005).

Indeed, sleep disturbances are frequently associated with PD, often preceding the motor onset of the disease itself and revealing the intimate role of DA in regulating the sleep-wake cycle (Dzirasa et al., 2006). Furthermore, improvement of motor performance in the morning is frequently mentioned by patients, principally in those with a long history of the disease and motor fluctuations (Bateman et al., 1999). Parallel to the disease progression, a progressive change in the physiological sleep architecture is observed, which includes a significant decrease in the amount of NREM sleep (Diederich et al., 2005; Rye and Jankovic, 2002).

In the present article, we investigated whether the development of LID might be associated with a critical impairment of synaptic downscaling during NREM, implying sustained retention of aberrant synaptic memories within the corticobasal ganglia-thalamic loop. To examine our hypothesis, we measured the synaptic strength in the following 5 animal groups: (i) sham-lesioned drug-naive rats (sham/LD$^-$), (ii) sham-lesioned LD-treated rats (sham/LD$^+$), (iii) 6-hydroxydopamine (6-OHDA)–lesioned drug-naive rats (PD/LD$^-$), (iv) 6-OHDA–lesioned LD-treated rats without dyskinesia (PD/DYS$^-$), and (v) 6-OHDA–lesioned LD-treated rats with dyskinesia (PD/DYS$^+$). We found that PD/DYS$^-$ animals had a physiological sleep-induced reduction of synaptic strength, whereas PD/DYS$^+$ animals do not manifest an adequate reduction in SWA, thus possibly implying reduced synaptic downscaling during sleep. Concordantly, behavioral experiments demonstrated that sleep deprivation (SD) was able to enhance the emergence of LID during LD treatment (Cam et al., 2013).

2. Methods

2.1. Animals

Experimental procedures were carried out on 142 adult male Sprague-Dawley rats (Harlan, Udine, Italy) weighing 170–200 g corresponding to ~6 weeks of age (Table 1, Fig. 1A and B). The study was conducted in compliance with Swiss laws on animal experimentation and the National Institute of Health's *Guide for the Care and Use of Laboratory Animals*. All procedures were performed minimizing animal discomfort and strain. Rats were maintained on a regular light-dark cycle (lights on at 10:00 AM, lights off at 10:00 PM; room temperature 20–22 °C; and maximum 3 animals per cage) and were given food (Harlan RM Diet; Harlan) and water ad libitum before habituation to the behavioral paradigm. The study design and the experimental groups are depicted in Fig. 1A. We employed a 6-OHDA–based parkinsonian rat model inducing abnormal involuntary movements (AIMs) comparable with LID observed in PD patients by chronic treatment with LD. The experimental procedures were conducted in the following 5 animal groups: (i) sham/LD$^-$, (ii) sham/LD$^+$, (iii) PD/LD$^-$, (iv) PD/DYS$^-$, and (v) PD/DYS$^+$. Electrophysiological and molecular experiments were performed in a blind manner in relation to assignment to the previously mentioned experimental groups.

2.2. Unilateral 6-OHDA–lesion model of PD

Unilateral (left hemisphere) DA denervation was performed according to a standard protocol (Galati et al., 2008; Schwarting and Huston, 1996). Briefly, rats were anaesthetized with 1.5%–2.5% isoflurane in oxygen and mounted on a stereotaxic instrument (Stoelting Co, Wood Dale, IL, USA). Body temperature was maintained at 37–38 °C with a heating pad (Stoelting Co) placed beneath the animal. After a subcutaneous injection of the local anesthetic bupivacaine, a midline scalp incision was made, and a hole (Ø of ~1.0 mm) was drilled in the skull on the left side. The neurotoxin (30-mM solution of 6-OHDA containing 0.03% of ascorbic acid) was injected into the medial forebrain bundle (coordinates: 4.0 mm posterior of the bregma, 1.3 mm laterally of the midline, and 7.0 mm beneath the cortical surface). Injections of 3 μL of 6-OHDA were administered through a 30-gauge cannula connected to a 10-μL Hamilton syringe over a period of 3 minutes. The injection of neurotoxin was preceded by a bolus of desipramine (25 mg/kg, intraperitoneally) to minimize the uptake of 6-OHDA by noradrenergic neurons. Two weeks later, an apomorphine-induced rotation test (0.05 mg/kg, subcutaneously) was performed to assess the severity of nigral lesions (Galati et al., 2008; Hudson et al., 1993). Animals performing at least 100 rotations opposite to the lesion site within 20 minutes from the apomorphine treatment (Hudson et al., 1993) were considered successfully lesioned and included in the study (Fig. 1B).

2.3. Induction of dyskinesia by chronic LD treatment

For the induction of LID, 6-OHDA–treated animals were submitted to LD treatment as described elsewhere (Fig. 1B and Cenci et al., 1998). Briefly, the rats were subcutaneously treated for 3 weeks with LD methyl ester (Sigma-Aldrich) at a daily dose of 8 mg/kg in combination with 15-mg/kg benserazide (Sigma-Aldrich). LID was rated 20 minutes after the injection according to a dyskinesia rating scale (Cenci et al., 1998). The rats were

Table 1
Groups of animals analyzed in this study ($n = 125$)

Electrophysiological and behavioral data ($n = 93$)	
Analysis of 24-h sleep recording and DARPP-32 level ($n = 30$ and $n = 6$ excluded)	Sham/LD$^-$ ($n = 6$)
	Sham/LD$^+$ ($n = 4$)
	PD/LD$^-$ ($n = 7$)
	PD/DYS$^-$ ($n = 6$)
	PD/DYS$^+$ ($n = 7$)
Analysis of LD washout on dyskinesia and SWA ($n = 8$)	PD/DYS$^+$ ($n = 8$)
Analysis of SD on dyskinesia and SWA ($n = 19$)	PD/DYS$^+$/SD$^+$ ($n = 6$)
	PD/DYS$^+$/SD$^-$ ($n = 4$)
	PD/DYS$^-$/SD$^+$ ($n = 4$)
	PD/DYS$^-$/SD$^-$ ($n = 5$)
Analysis of CS-EP ($n = 30$)	Sham/LD$^-$ ($n = 10$)
	PD/DYS$^-$ ($n = 10$)
	PD/DYS$^+$ ($n = 10$)
Molecular data ($n = 32$)	
Analysis of cortical Arc gene expression ($n = 32$)	Sham/LD$^-$ ($n = 4$)
	Sham/LD$^+$ ($n = 4$)
	PD/LD$^-$ ($n = 4$)
	PD/DYS$^-$ ($n = 10$)
	PD/DYS$^+$ ($n = 10$)
Analysis of cortical Arc protein level ($n = 26$)	Sham/LD$^-$ ($n = 4$)
	Sham/LD$^+$ ($n = 4$)
	PD/LD$^-$ ($n = 5$)
	PD/DYS$^-$ ($n = 5$)
	PD/DYS$^+$ ($n = 8$)
Analysis of striatal Arc gene expression ($n = 24$)	Sham/LD$^-$ ($n = 4$)
	Sham/LD$^+$ ($n = 4$)
	PD/LD$^-$ ($n = 4$)
	PD/DYS$^-$ ($n = 5$)
	PD/DYS$^+$ ($n = 7$)
Analysis of striatal Arc protein level ($n = 25$)	Sham/LD$^-$ ($n = 4$)
	Sham/LD$^+$ ($n = 4$)
	PD/LD$^-$ ($n = 4$)
	PD/DYS$^-$ ($n = 5$)
	PD/DYS$^+$ ($n = 8$)

Key: CS-EP, corticostriatal-evoked potential; LD, levodopa; PD/LD$^-$, 6-hydroxydopamine (6-OHDA)–lesioned drug-naive rats; PD/DYS$^-$, 6-OHDA–lesioned LD-treated rats without dyskinesia; PD/DYS$^+$, 6-OHDA–lesioned LD-treated rats with dyskinesia; SD, sleep deprivation; sham/LD$^+$, sham-lesioned LD-treated rats; sham/LD$^-$, sham-lesioned drug-naive rats; SWA, slow-wave activity.

observed in individual cages every 20 minutes for 3 hours, and AIMs were scored according to a scale from 0 to 4 for each of the following 4 categories: (1) limb dyskinesia, (2) axial dystonia, (3) orolingual movements, and (4) rotational, locomotor behavior. "0" was assigned in the absence of abnormal movements, "1" indicated the presence of dyskinesia occurring for less than half the observational period, "2" was attributed when dyskinetic movements occurred for more than half the observational period, "3" indicates a condition in which constant dyskinesia is only briefly interrupted, and "4" refers to constant uninterruptible dyskinesia. The behavioral assessment yielded a subdivision of chronically LD-treated rats into dyskinetic and non-dyskinetic subgroups.

2.4. Implantation of EEG electrodes

All animals submitted to 24-hour sleep recording (Table 1, Fig. 1A) were implanted with EEG electrodes at 10 weeks of age (Fig. 1B and C). Gold screw electrodes were implanted bilaterally in frontal cortex (coordinates: 2 mm anterior of the bregma and 3 mm lateral to the midline in the left and right frontal skull) and parietal cortex (coordinates: 2.5 mm posterior of the bregma and 5.5 mm lateral to the midline in the left and right parietal skull); one reference epidural electrode was placed over the left cerebellar hemisphere (Fig. 1C). Electromyogram (EMG) was recorded by 2 stainless steel wires (Ø of 0.3 mm and ~30 mm of length) inserted bilaterally into the neck muscles. All electrodes were connected to stainless steel wires soldered to a plug subsequently fixed to the skull with dental cement. After 1 week, EEG recordings were performed for 24 hours preceded by 48 hours of habituation (Fig. 1B).

2.5. Electrophysiological data analysis

The EEG and EMG signals were amplified (×2000), sampled at 200 Hz (AURA-LTM64; Grass Telefactor) and saved to a computer for subsequent analysis. The EEG signal was digitally filtered post-acquisition (Notch type, 50 Hz; low-frequency filter, 1 Hz, and high-frequency filter, 30 Hz). Determination of arousal state was made off-line by visual scoring of EEG and EMG in 10-second epochs using a dedicated software (Somnologica; Embla Systems Inc). The arousal state was classified as NREM sleep, REM sleep, or wake on the basis of state-dependent changes. During the assignment of the arousal state, any epoch containing movement artifacts or electrical noise was tagged and excluded from subsequent spectral analyses.

EEG signals from the recorded derivations were downsampled to 100 Hz using the Matlab resample routine and filtered (0.3–70 Hz) using a third-order Butterworth filter. Spectral density analysis with a 0.1-Hz bin resolution was performed (Welch averaged modified periodogram with a Hamming window, applied on 10-second epochs). SWA (average spectral density between 0.5 and 4 Hz) was computed for each 10-second NREM epoch and then normalized by the average SWA across all NREM epochs in the 24-hour recording time (Esser et al., 2007).

We compared the average SWA of early (during the first hour after the beginning of the light period) and late (during the last hour of the light period) NREM sleep as an electrophysiological proxy of the homeostatic reduction of cortical synaptic strength,

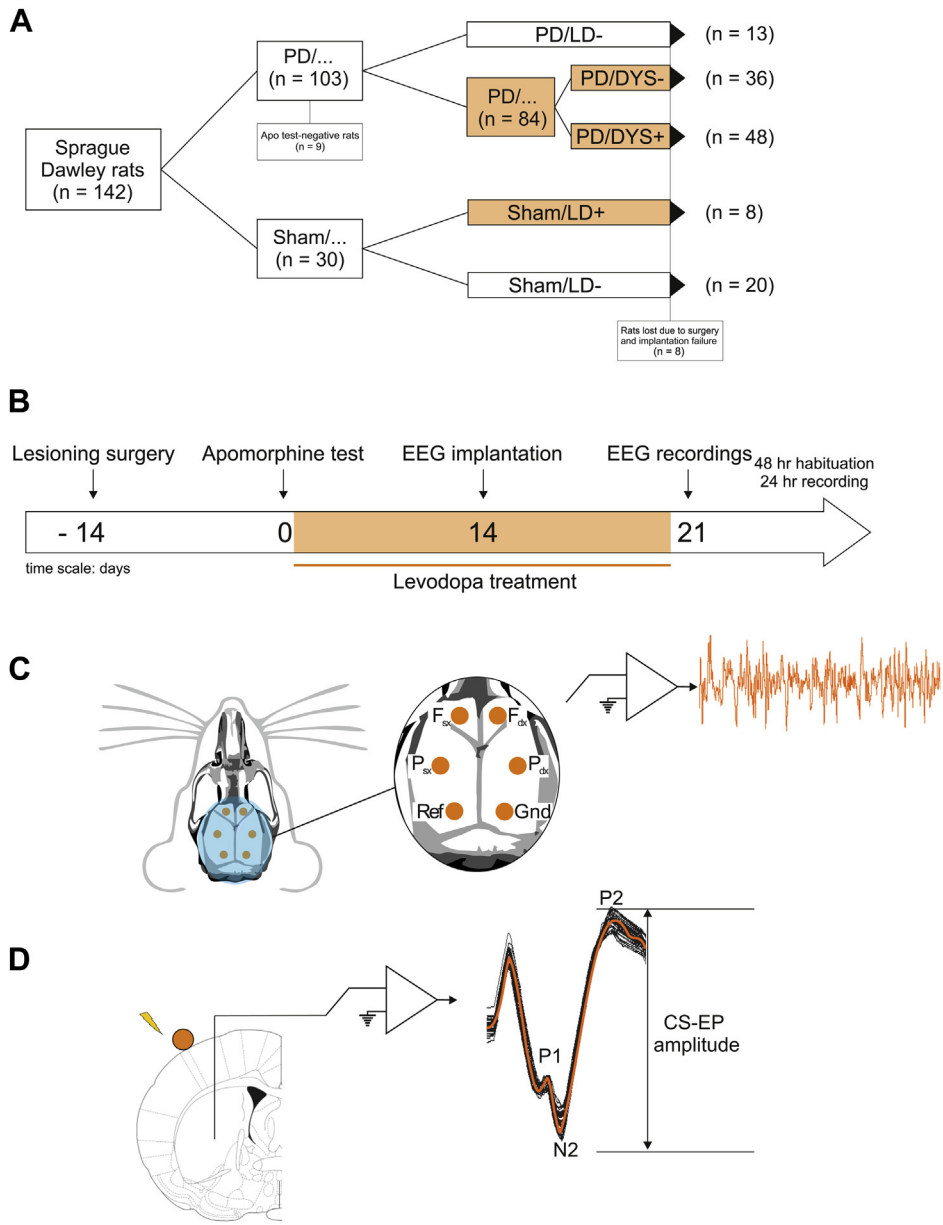

Fig. 1. Methodological approach for obtaining the animal groups and electrophysiological recordings. (A) Synopsis of the animals used in the study. After 6-hydroxydopamine (6-OHDA) or saline injection, we obtained 2 populations, the Parkinson's disease (PD)/…and sham/… groups, respectively. These 2 categories were then submitted or not to levodopa (LD) treatment (marked in orange) by which it was possible to obtain all used animal groups. (B) Time course of the procedures beginning with the lesioning surgery injecting 6-OHDA, the apomorphine challenge (to assess the severity of lesion), the LD treatment (for those animals that received this drug), the electroencephalogic implantation, and recordings or perfusion for those animals subjected to molecular analysis. (C) Not-in-scale representation of the placement of the screw electrodes over the frontal and the parietal cortices (orange circles) with a magnified view of the skull area in transparent blue. Ref. = reference and Gnd = ground. (D) Schematic representation of the method applied to perform and analyze corticostriatal-evoked potential (CS-EP). Coronal brain section 3.0 mm posterior to bregma. Orange circle represents the stimulating electrode over the left frontal cortex. Black line depicts the recording electrode within the left striatum.

that is, synaptic downscaling. Furthermore, we performed an LD washout in PD/DYS^{+} rats (n = 8, Table 1) more than 7 days to analyze recovery of synaptic downscaling. The dyskinesia score was assessed before and after the washout period as described previously.

2.6. SD protocol

SD began at light onset and was performed for 4 hours by exposing the animals to a variety of novel objects and the transfer of bedding material between cages. If rats (n = 10) became inactive and began to exhibit slow waves visible in real-time EEG monitoring (performed in 8 rats), a new object was inserted into the cage, and the cage was tapped or the rat was gently prodded. Rats were not disturbed when they were spontaneously awake and active (Cam et al., 2013). The animals were subjected to this SD every other day for 1 week. SWA analysis was performed during the light period before SD and in the interval between SD as described earlier, whereas behavioral assessment was performed 3 times a week.

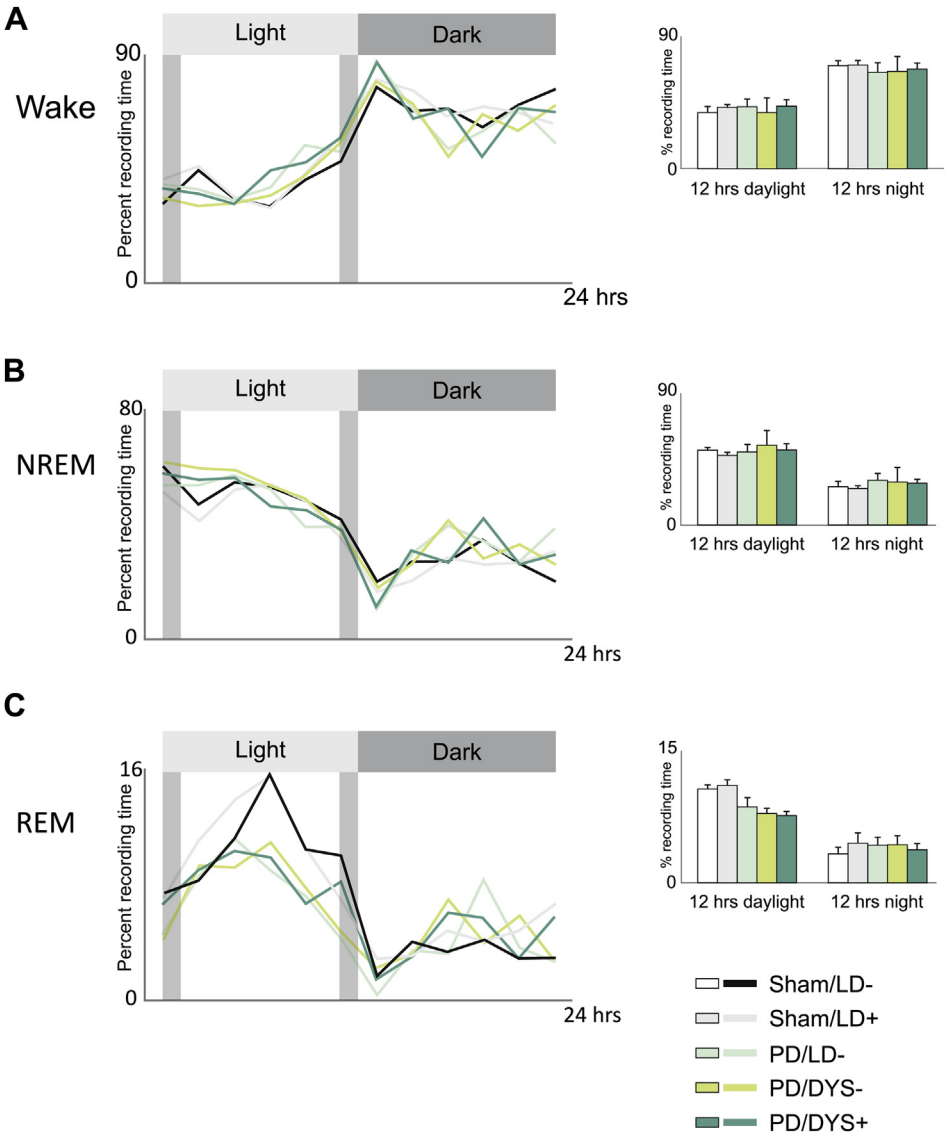

Fig. 2. Sleep scoring of the 5 groups of animals: (i) sham-lesioned drug-naive rats (sham/levodopa [LD]$^-$) ($n = 6$), (ii) sham-lesioned LD-treated rats (sham/LD$^+$) ($n = 4$), (iii) 6-hydroxydopamine (6-OHDA)–lesioned drug-naive rats (PD/LD$^-$) ($n = 7$), (iv) 6-OHDA–lesioned LD-treated rats without dyskinesia (PD/DYS$^-$) ($n = 6$), and (v) 6-OHDA–lesioned LD-treated rats with dyskinesia (PD/DYS$^+$) ($n = 7$). (A–C) Percent recording time for wake, nonrapid eye movement (NREM) sleep, and REM sleep from the beginning of the light period with intervals of 2 hours for each animal group. The transparent bars in the light period represent the early-sleep and late-sleep periods subjected to analysis. The insets display the percent recording time averaged for the 12-hour light and dark periods for the 3 conditions. No statistical difference between sham/LD$^-$ and the other groups was found (each $p > 0.05$, paired t test). The sham/LD$^-$ group is represented as black line in the linear plots and as white box in the box plots. Data are expressed as mean ± standard error of the mean.

2.7. Measurement of corticostriatal-evoked potentials

Electrical stimulation of the left cortical cortex evoked a complex striatal field potential that typically consisted of a positive-negative-positive (P1-N2-P2) waveform (Fig. 1D). To assess corticostriatal synaptic strength (Galiñanes et al., 2011), we measured these corticostriatal-evoked potentials (CS-EPs). For that purpose, screw stimulating electrodes were implanted into the left frontal cortex as described previously. Moreover, a wire recording electrode (22 μm, Stablohm 650; California Fine Wire, Grover Beach, CA, USA) was implanted into the left striatum (coordinates: 0.3 mm posterior of the bregma, 3.5 mm lateral to the midline, and 5.0 mm ventral to the cortical surface). A Grass stimulator was used to apply a basic stimulation consisting of 50 stimuli (impulse duration 0.1 μs, 0.4 Hz, voltage from 5 to 12 V to achieve the highest CS-EP amplitude possible). Subsequently, 3 trains of high-frequency stimulation (100 Hz) with a duration of 3 seconds and an interval of 20 seconds were applied followed by a low-frequency stimulation at 2 Hz for 10 minutes. The recording of CS-EP was performed in the awake animal during the first and the last hours of the light period. The cortical stimulation parameters in early and late sleep were identical for each animal.

The amplitude of CS-EP was determined as the voltage difference between the peaks N2 and P2. The signal was referenced to a screw electrode in the occipital bone, amplified, band-pass filtered (1 and 300 Hz), digitized (10 kHz), and stored in a computer for off-line analysis.

2.8. Molecular biology experiments

The animals were euthanized, and the brains were removed and stored in RNAlater (Sigma-Aldrich) at −80 °C for subsequent analysis. The expression of Arc on the messenger RNA (mRNA) and protein level in both cortex and striatum and the content of

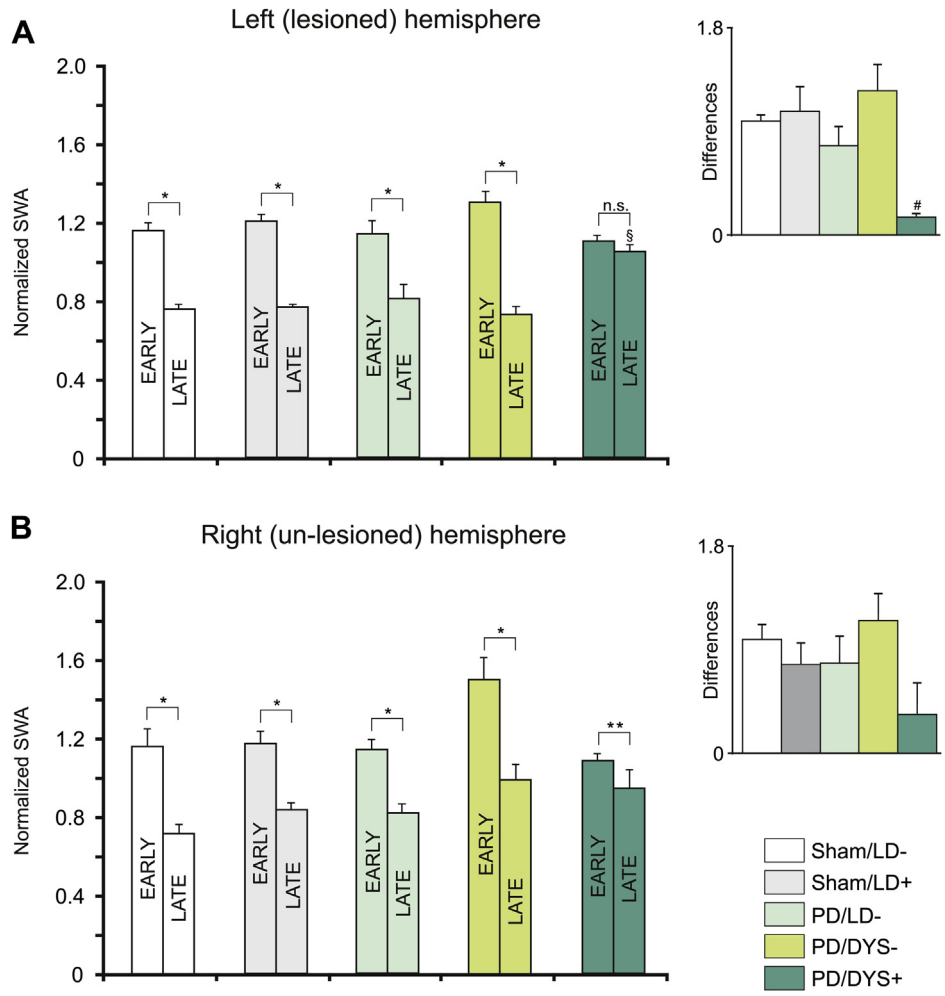

Fig. 3. Effect of sleep on slow-wave activity (SWA) measured at the beginning (early sleep) and at the end of the light period (late sleep) in the 5 groups of animals. (A) Results over the lesioned hemisphere. SWA during early and late nonrapid eye movement (NREM) sleep was compared with each other in the 5 animal groups. All groups showed a physiological SWA reduction except for the 6-hydroxydopamine (6-OHDA)–lesioned levodopa (LD)–treated rats with dyskinesia (PD/DYS$^+$) group that did not feature a significant difference between early and late NREM sleep ($n = 7$, $p > 0.05$). The comparison of the differences between groups (inset on the right) showed a clearly lower difference in the PD/DYS$^+$ group than in the other groups. Of note, the 6-OHDA–lesioned LD-treated rats without dyskinesia (PD/DYS$^-$) ($n = 6$) group featured a slightly but not significantly higher SWA difference in comparison with the sham groups and 6-OHDA–lesioned drug-naive rats (PD/LD$^-$) animals ($n = 7$, $p > 0.05$). (B) In the right unlesioned hemisphere, the comparison between early and late sleep was significant in all groups, including the PD/DYS$^+$ group ($n = 7$, $p < 0.05$). As a consequence, the comparison of the differences between groups did not show any significance (inset on the right). *$p < 0.001$ early versus late sleep; **$p < 0.05$ early versus late sleep; ns, not significant; §$p < 0.05$ versus all groups; and #$p < 0.001$ versus all groups. Data are expressed as mean ± standard error of the mean.

Thr34-phosphorylated-DARPP-32 (P-Thr34-DARPP-32) and unphosphorylated DARPP-32 within the striatum were assessed.

2.8.1. Real-time polymerase chain reaction

Total RNA was extracted from samples of cortex and striatum, using a Qiagen RNeasy Microarray Tissue Mini Kit and then quantified using a NanoDrop 2000c spectrophotometer. The complementary DNA was obtained from up to 2 μg of total RNA by using a high-capacity RNA-to-cDNA kit (Invitrogen) and stored at −20 °C. 5′-fluorescein amidite–labeled probes were used in the TaqMan real-time quantitative reverse transcription-polymerase chain reaction assay for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (endogenous control, assay ID: Rn01775763_g1) and Arc (assay ID: Rn00571208_g1) (both Applied Biosystems, Foster City, CA, USA). Reactions were performed in triplicate on an AB 7900HT fast real-time PCR system (Applied Biosystems). Relative quantification of Arc mRNA was determined using the ΔΔCT method, where CT represents threshold cycle, as described in detail elsewhere (Béïque et al., 2011). Briefly, triplicate values of CT, cycle at which the sample reached the threshold fluorescence level) for each sample were averaged. Mean CT values for GAPDH were subtracted from mean CT values for Arc for each sample resulting in ΔCT. The highest ΔCT from the sham/LD group was used as a calibrator and then subtracted from the ΔCT of the remaining samples (ΔΔCT, Livak and Schmittgen, 2001). The fold change in the expression of Arc, normalized for GAPDH, was then determined using the formula $2 - \Delta\Delta CT$. These values were subsequently averaged for each of the 4 groups.

2.8.2. Western blot

Cortical and striatal samples were homogenized in ice-cold Tissue Extraction Reagent I (Invitrogen) with addition of 0.5% protease inhibitor cocktail (Sigma, St Louis, MO, USA) and 1% phosphatase inhibitor cocktail (Thermo Scientific, Rockford, IL, USA) followed by sonication and centrifugation. The protein concentration of the supernatants was determined using a Micro BCA Protein Assay kit (Thermo Scientific) according to the manufacturer's instructions. Equal amounts of samples (40 μg of protein) were loaded onto Mini-Protean TGX Precast Gels (Bio-Rad) for gel electrophoresis and then transferred to a 0.2-μm

nitrocellulose membrane (Ready-Blot Sandwiches; Bio-Rad). The membrane was then blocked in Tris-buffered saline and Tween 20 (TBS-T) buffer with 5% bovine serum albumin for 1 hour at room temperature. The membranes were subsequently incubated at 4 °C overnight with the following primary antibodies diluted in TBS-T + 2.5% bovine serum albumin: mouse anti-Arc monoclonal antibody (1:1000; BD Bioscience), rabbit anti-DARPP-32 and rabbit anti-P-Thr34-DARPP-32 polyclonal antibodies (1:2000; Cell Signaling Technology, Danvers, MA, USA), and mouse anti-GAPDH monoclonal antibody (1:3000; Abcam). After 3× 10-minute washes in TBS-T, membranes were incubated with the appropriate secondary horseradish peroxidase–linked antibodies (1:3000) for 1 hour at room temperature. Protein bands were visualized using a ChemiGlow West Chemiluminescence Substrate Kit (Bucher Biotec), and band immunoreactivity was analyzed by ImageQuant LAS 4000 (GE Healthcare Life Sciences). Integrated optical density of bands was measured using the software ImageJ to generate quantitative data of band intensities. The optical density was normalized to the background values. Values were corrected using GAPDH values as a reference.

2.9. Statistical analyses

All statistical analyses were performed with SPSS (PASW, version 17). Comparisons of normalized SWA between early and late sleep were performed by Mann-Whitney *U* test. Between-group analyses were performed by Kruskal-Wallis test followed by Mann-Whitney *U* test for post hoc analyses. Gene expression differences were analyzed by *t* test. Multiple comparisons were corrected by Bonferroni test. The correlation analyses between the P-Thr34-DARPP-32/DARPP-32 ratio and SWA differences and arc protein levels, respectively, was performed using nonparametric Spearman correlation. Also, the Mann-Whitney *U* test was used for the comparison of the P-Thr34-DARPP-32/DARPP ratio in PD/DYS$^-$ and PD/DYS$^+$ groups. For statistical analysis of corticostriatal-evoked potentials and SWA during SD, we used Mann-Whitney *U* test for between-group analysis and analysis of variance according to the Friedman for within-group analysis followed by Wilcoxon signed-rank test for post hoc analysis. For the analysis of SWA during LD washout, we used Mann-Whitney *U* test.

3. Results

For the electrophysiological and behavioral experiments, 93 out of a total of 125 rats were used (Table 1, Fig. 1A and B). Of these, 36 rats were submitted to 24-hour sleep recording after a 48-hour habituation, and 6 recordings (2 PD/LD$^-$, 1 PD/DYS$^-$ and 3 PD/DYS$^+$) were excluded from subsequent evaluation because of poor quality of the signal from at least 2 out of the 4 scalp electrodes (Table 1, Fig. 1C). Finally, the following animals were included in the electrophysiological analysis: (i) sham/LD$^-$ ($n = 6$), (ii) sham/LD$^+$ ($n = 4$), (iii) PD/LD$^-$ ($n = 7$), (iv) PD/DYS$^-$ ($n = 6$), and (v) PD/DYS$^+$ ($n = 7$). All animals slept preferentially during the light period ($n = 30$, waking, 40.4% ± 2.33%; NREM sleep, 50.4% ± 3.09%; and REM sleep, 9.1% ± 0.6%) compared with the dark period (waking, 68.3% ± 2.9%; NREM sleep, 27.6% ± 2.47%; REM sleep, 3.9% ± 0.4%; and light vs. dark, all $p <$ 0.001, data not shown). No significant changes of the average recording time for each vigilance state (wake, NREM sleep, and REM sleep) were observed among the groups (each $p > 0.05$, Fig. 2A–C).

3.1. PD/DYS$^+$ animals have an impaired reduction of SWA during NREM sleep

As a result of the asymmetric nature of the 6-OHDA parkinsonian animal model, we systematically conducted our analysis in both hemispheres, that is, the lesioned one on the left (Fig. 3A) and the unlesioned one on the right (Fig. 3B).

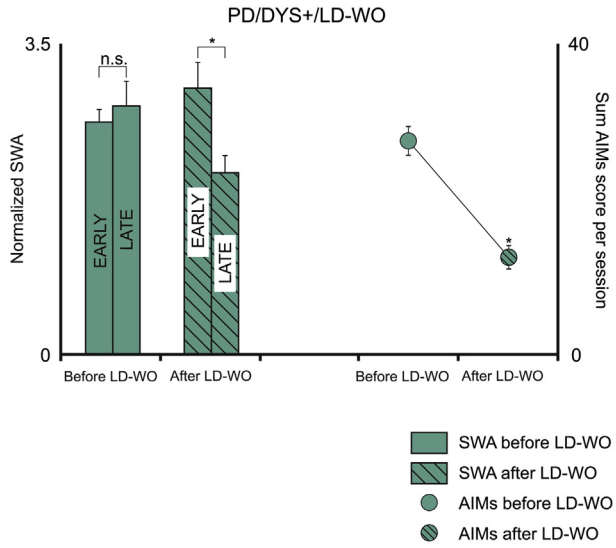

Fig. 4. One-week levodopa (LD) washout. The plot illustrates both the slow-wave activity (SWA) (on the left) and the abnormal involuntary movement (AIM) (on the right) scores of 6-hydroxydopamine (6-OHDA)–lesioned LD-treated rats with dyskinesia (PD/DYS$^+$) animals ($n = 8$) before and 1 week after LD washout. PD/DYS$^+$ animals had a recovery of physiological SWA reduction after LD washout ($p < 0.05$) with a parallel clear-cut decrease of the AIM scores ($p < 0.05$). The shaded vertical bars and circle represent SWA and AIMs after LD washout, respectively. LD-WO, LD washout; $^*p < 0.05$ early versus late sleep; and ns, not significant. Data are expressed as mean ± standard error of the mean.

During NREM sleep, SWA shows a physiological decline (Hanlon et al., 2009; Vyazovskiy et al., 2007). Accordingly, our control animals (sham/LD$^-$) showed a significantly lower late-sleep SWA (Fig. 3A and B, $p < 0.001$) compared with early sleep, suggesting an appropriate synaptic downscaling (Hanlon et al., 2009; Vyazovskiy et al., 2007). With respect to the lesioned hemisphere on the left, we found that all groups showed this physiological reduction of SWA except for the PD/DYS$^+$ rats, in which the difference between early and late sleep was not significant ($p > 0.05$, Fig. 3A). Moreover, the PD/DYS$^+$ animals showed a higher late-sleep SWA than the other groups (each $p < 0.05$, Fig. 3A). Accordingly, PD/DYS$^+$ animals had a significantly reduced difference between early-sleep and late-sleep SWA compared with the other groups (each $p < 0.001$, Fig. 3A, inset). PD/DYS$^-$ animals showed a tendency to a higher SWA difference between early and late sleep in comparison with the PD/LD$^-$ group but without reaching statistical significance ($p = 0.07$, Fig. 3A).

The right unlesioned hemisphere showed a significant decline of SWA in all groups (PD/DYS$^+$ rats, $p < 0.05$ and all others, $p < 0.001$, Fig. 3B). Although PD/DYS$^+$ rats showed descriptively the lowest difference between early-sleep and late-sleep SWA, this difference was not significant in comparison with the sham and PD/LD$^-$ groups ($p > 0.05$, Fig. 3B, inset). PD/DYS$^-$ animals showed a tendency to higher SWA during early sleep in comparison with the other groups of animals (Fig. 3B).

3.2. Effects of LD washout and SD on dyskinesia and SWA

To corroborate our previous findings, we performed a 1-week LD washout in a group of additional PD/DYS$^+$ animals ($n = 8$, Table 1). As shown in Fig. 4, we observed a significant recovery of the reduction of SWA in late compared with early sleep ($p < 0.05$). In analogy to the electrophysiological data, we also found a significant decrease of AIMs ($p < 0.05$).

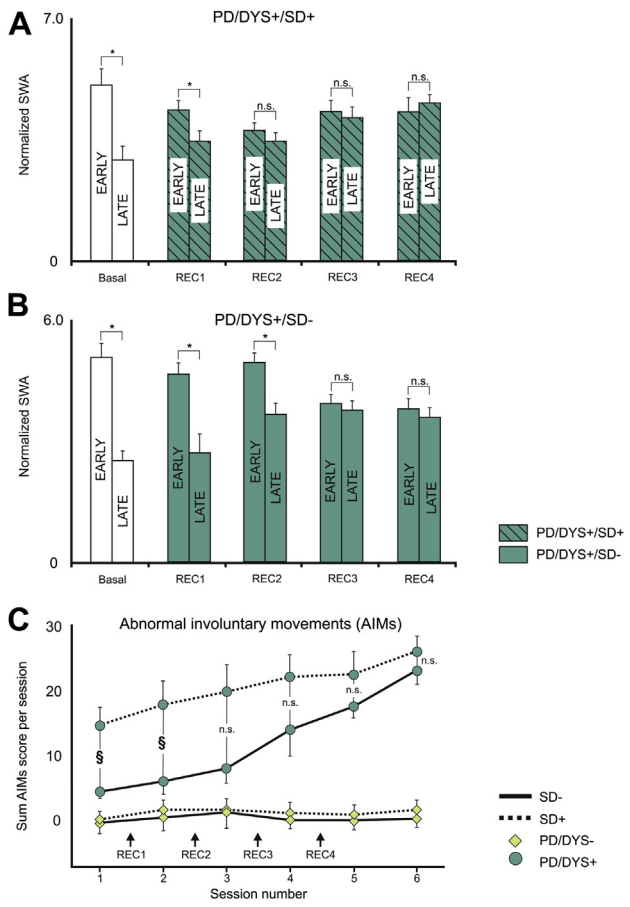

Fig. 5. Electrophysiological and behavioral effects of sleep deprivation (SD). (A) Slow-wave activity (SWA) in early and late sleep before starting levodopa (LD) treatment (basal) and during simultaneous performance of the SD and LD treatment protocols in 6-hydroxydopamine (6-OHDA)–lesioned LD-treated rats with dyskinesia (PD/DYS$^+$)/SD$^+$ rats. We found a progressive decrease of the difference between early-sleep and late-sleep SWA during the observational period of 2 weeks (shaded green vertical bars). The lack of a significant difference between early-sleep and late-sleep SWA was reached on REC2 corresponding to 4 days after the initiation of the SD and LD treatment protocols. (B) SWA in early and late sleep before starting LD treatment (basal) and during LD treatment in PD/DYS$^+$/SD$^-$ rats. As expected, we found a progressive decrease of the difference between early-sleep and late-sleep SWA. In comparison with PD/DYS$^+$/SD$^+$ rats, the lack of a significant difference between early-sleep and late-sleep SWA was reached on REC3 corresponding to 6 days after the initiation of LD therapy (green vertical bars). (C) Effect of SD on the emergence of dyskinesia as measured by the abnormal involuntary movement (AIM) scale. As shown, SD significantly anticipated the occurrence of dyskinesia by approximately 3 days (green circles, dashed line, $n = 6$) in comparison with animals not subjected to SD (green circles, continuous line; $n = 4$). Regardless of the SD protocol, a subpopulation of animals (SD$^+$, $n = 4$; SD$^-$, $n = 5$) did not show dyskinesia during LD therapy (yellow rhombus, dashed and continuous line, SD$^+$ and SD$^-$, respectively). REC1, 2 days and REC4, 8 days after initiation of SD. *$p < 0.05$ early versus late sleep; ns, not significant; and §$p < 0.05$ PD/DYS$^+$/SD$^+$ versus PD/DYS$^+$/SD$^-$. Data are expressed as mean ± standard error of the mean.

Simultaneously to the beginning of LD therapy, we subjected another 10 parkinsonian rats to a 2-week SD, whereas 9 parkinsonian rats served as controls (Table 1). We obtained the following groups: PD/DYS$^+$/SD$^+$ ($n = 6$), PD/DYS$^+$/SD$^-$ ($n = 4$), PD/DYS$^-$/SD$^+$ ($n = 4$), and PD/DYS$^-$/SD$^-$ ($n = 5$). As shown in Fig. 5A, we observed an anticipation of the lack of the physiological reduction of SWA in sleep-deprived animals that became evident as early as day 4 (REC2 in Fig. 5A and B). In agreement with the electrophysiological data, we observed that the occurrence of dyskinesia was significantly anticipated by approximately 3 days in the SD$^+$ animals (PD/DYS$^+$/SD$^+$, $n = 6$; PD/DYS$^+$/SD$^-$, $n = 4$; Fig. 5C). After 8 days (REC4 in Fig. 5), the difference between the 2 groups (PD/DYS/SD$^+$ and PD/DYS/SD$^-$) with respect to SWA and the AIM scale was no longer significant.

3.3. Cortical Arc gene expression and protein level are increased in PD/DYS$^+$ rats

Thirty-two out of 125 animals (Table 1, Fig. 1A and B) were used for subsequent molecular analysis. We chose the same time of the day, that is, before light ending at 10:00 PM corresponding to the beginning of the wake period. Because Arc is directly proportional to induction and maintenance of LTP in dendritic spines (Messaoudi et al., 2007) and physiologically decreases during sleep (Hanlon et al., 2009), we determined its expression at the mRNA and protein levels within the frontal cortex and the striatum to explore how sleep modifies its expression in the 5 groups of animals.

As far as the cortical Arc mRNA expression is concerned, we compared its levels with that of the highest (ΔCT) value of the sham/LD$^-$ group ($n = 4$, for details, see "Methods"). The Arc mRNA level was 1.50 ± 0.07 times higher in the PD/DYS$^+$ group ($n = 10$, Fig. 6A), that is, significantly different from the PD/LD$^-$ group that showed a clear decrease of the Arc mRNA content ($n = 4$, 0.44 ± 0.10-fold, $p < 0.001$, Fig. 6A). The decrease of Arc gene expression was even more pronounced in PD/DYS$^-$ animals ($n = 10$, 0.08 ± 0.04-fold, $p < 0.001$, Fig. 6A). However, the Arc mRNA level of the PD/DYS$^+$ animals was not significantly different from the sham/LD$^+$ rats ($n = 4$, 1.21 ± 0.01-fold, $p > 0.01$, Fig. 6A). Of note, we found a significant difference between the sham/LD$^+$ and the PD/LD$^-$ rats ($p < 0.01$, Fig. 6A). The latter group was also significantly different from the PD/DYS$^-$ animals ($p < 0.01$, Fig. 6A). We further extended the analysis of Arc gene expression to a comparison of the 2 hemispheres in PD/DYS$^+$ animals, that is, the left lesioned side and the right unlesioned side. We found a significant difference in Arc gene expression between the 2 hemispheres with a clear-cut increase on the lesioned side (1.50 ± 0.18-fold vs. 0.85 ± 0.16-fold of the unlesioned hemisphere, $p < 0.01$, Fig. 6A, inset).

In agreement with the previous results, the Arc protein levels within cortex were higher in PD/DYS$^+$ rats ($n = 8$, each $p < 0.01$, Fig. 6B) than in the other groups of animals. Comparable with the mRNA results, the Arc protein level was not significantly different between the sham/LD$^-$ ($n = 4$) and sham/LD$^+$ ($n = 4$) rats ($p > 0.05$, Fig. 6B). However, we did not find any statistical difference between PD/LD$^-$ ($n = 5$) and PD/DYS$^-$ ($n = 5$) rats ($p > 0.05$, Fig. 6B) that were both slightly increased compared with the 2 sham groups ($p > 0.05$, Fig. 6B). The additional interhemispheric analysis of the PD/DYS$^+$ rats showed, in agreement with the mRNA data, a statistically significant increase of the Arc protein on the lesioned side ($p < 0.001$, Fig. 6B, inset).

3.4. Striatal Arc gene expression and protein levels are increased in PD/DYS$^+$ rats

We further explored the Arc gene expression and its protein level within the striatum. We found a significant increase of Arc mRNA levels in the PD/DYS$^+$ ($n = 7$), PD/DYS$^-$ ($n = 5$), PD/LD$^-$ ($n = 4$), and sham/LD$^+$ ($n = 4$) groups in comparison with the sham/LD$^-$ ($n = 4$) group. The Arc mRNA level was ~2.5 times higher in PD/DYS$^+$ than in sham/LD$^-$ animals ($p < 0.05$, Fig. 7B). Both the PD/DYS$^-$ and the PD/LD$^-$ rats showed a significant 2.0- and 1.5-fold increase of the striatal Arc mRNA expression, respectively (all $p < 0.05$, Fig. 7A). In agreement with the cortex results, no significant difference was observed between sham/LD$^+$ and sham/LD$^-$animals (~1.2-fold, $p > 0.05$, Fig. 7A). The analysis of Arc gene expression between the 2 hemispheres in PD/DYS$^+$ animals showed a

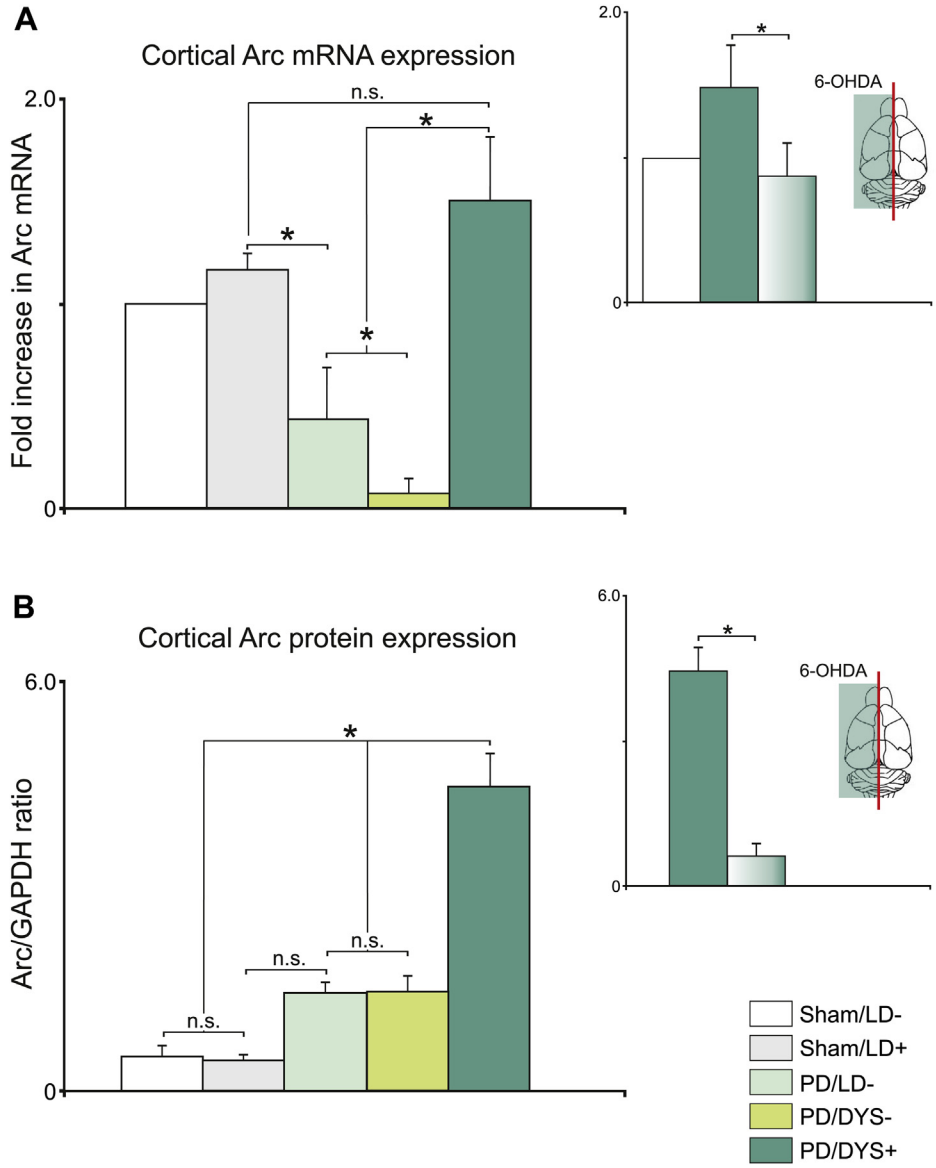

Fig. 6. Cortical Arc gene and protein expression. (A) The bar plot illustrates the difference of the quantitative polymerase chain reaction analysis of Arc messenger RNA (mRNA) in comparison with the sham-lesioned drug-naive rats (sham/levodopa [LD]$^-$) ($n = 4$) group. 6-hydroxydopamine (6-OHDA)–lesioned LD-treated rats with dyskinesia (PD/DYS$^+$) ($n = 10$) animals had a significantly higher Arc mRNA expression ($p < 0.05$). The differences were also significant between the 6-OHDA–lesioned LD-treated rats without dyskinesia (PD/DYS$^-$) ($n = 10$) and 6-OHDA–lesioned drug-naive rats (PD/LD$^-$) ($n = 4$) and between the PD/LD$^-$ and sham-lesioned LD-treated rats (sham/LD$^+$) ($n = 4$) groups. On the contrary, no difference was observed between sham/LD$^+$ and sham/LD$^-$ animals ($p > 0.05$). The inset on the right shows the significant interhemispheric difference between the left lesioned (6-OHDA) and right unlesioned hemispheres (represented by white-green blending color) within the PD/DYS$^+$ group. (B) Cortical Arc protein expression in the 5 animal groups: sham/LD$^-$ ($n = 4$), sham/LD$^+$ ($n = 4$), PD/LD$^-$ ($n = 5$), PD/DYS$^-$ ($n = 5$), and PD/DYS$^+$ ($n = 8$). In agreement with the gene expression, we found a significant difference between the PD/DYS$^+$ animals in comparison with all the other groups ($p < 0.05$). We found a similar interhemispheric difference between the left lesioned (6-OHDA) and right unlesioned hemisphere (represented by white-green blending color) within the PD/DYS$^+$ group ($p < 0.05$). *$p < 0.001$ each; data are expressed as mean ± standard error of the mean.

significant increase of the Arc mRNA on the left side in comparison with the right side (~1.90 fold, $p < 0.001$, Fig. 7A, inset).

The Arc protein levels within the striatum mirrored the gene expression results. Thus, we found that striatal Arc levels were higher in PD/DYS$^+$ rats ($n = 8$) than in all the other groups ($p < 0.05$, Fig. 7B). Although significantly lower than in dyskinetic animals, PD/DYS$^-$ animals ($n = 5$, $p < 0.05$, Fig. 7B) showed a tendency to higher but not significantly increased levels of Arc protein ($p > 0.05$, Fig. 7B). Similarly, no difference was observed between sham/LD$^-$ and sham/LD$^+$ rats ($p > 0.05$, Fig. 7B). In addition, we observed an interhemispheric difference with respect to the striatal level of Arc protein in the PD/DYS$^+$ animals ($p < 0.05$, Fig. 7B, inset).

3.5. Synaptic downscaling inversely correlates with the striatal P-Thr34-DARPP-32 levels

It has been demonstrated that animals showing dyskinesia have significantly higher levels of P-Thr34-DARPP-32 than nondyskinetic animals (Picconi et al., 2003). This augmentation seems to be directly correlated with the lack of depotentiation observed in dyskinetic rats because of its relation to an inhibition of the protein phosphatase 1, as demonstrated in hippocampus (Hemmings et al., 1984).

Consistently, we found a significant increase of the striatal P-Thr34-DARPP-32/DARPP-32 ratio ($p < 0.05$, Fig. 8A) in PD/DYS$^+$

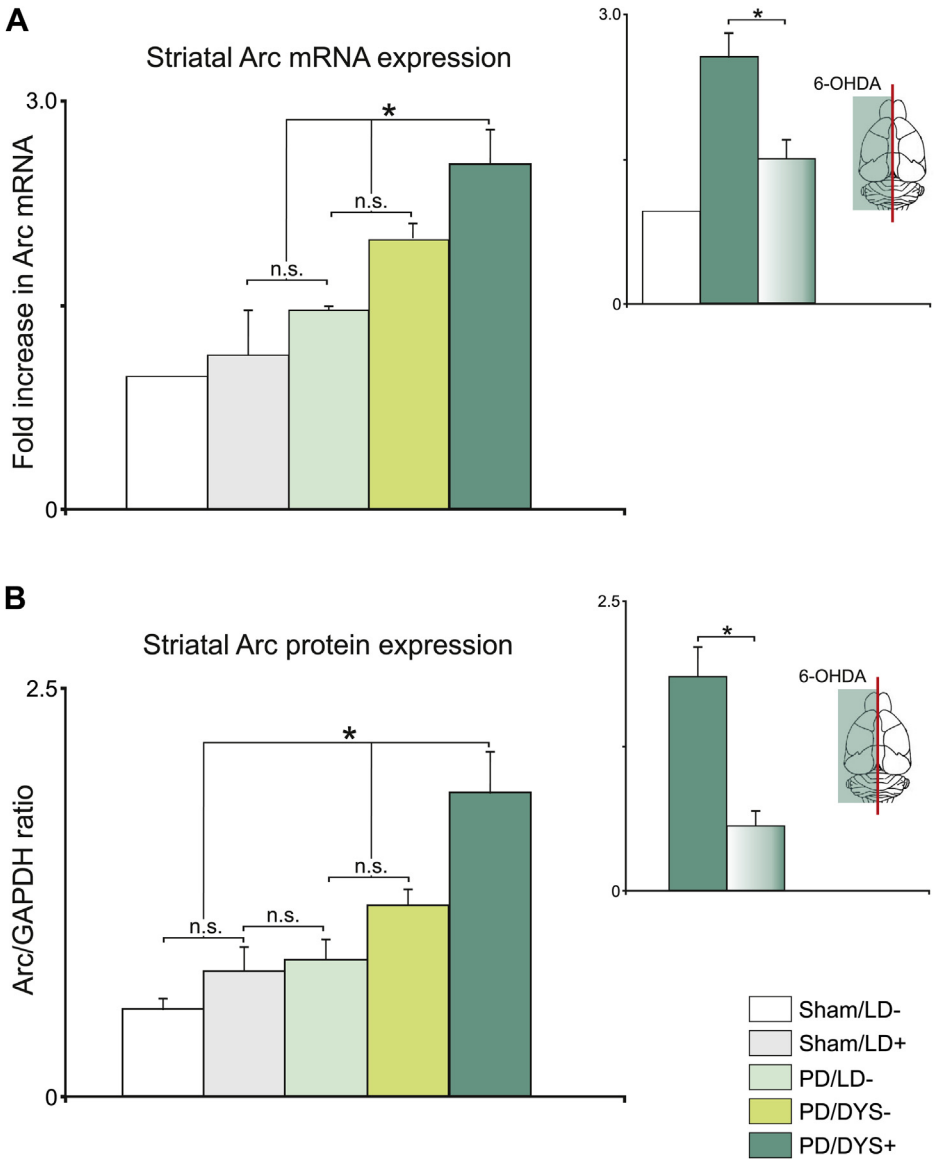

Fig. 7. Striatal Arc gene and protein expression. (A) Difference of the quantitative polymerase chain reaction analysis of Arc messenger RNA (mRNA) in striatum in comparison with the sham-lesioned drug-naive rats (sham/levodopa [LD]$^-$) group ($n = 4$). In analogy to the cortical findings, 6-hydroxydopamine (6-OHDA)–lesioned LD-treated rats with dyskinesia (PD/DYS$^+$) animals had a significantly higher Arc mRNA ($n = 7$, $p < 0.05$). However, no significant differences were found among sham-lesioned LD-treated rats (sham/LD$^+$) ($n = 4$), 6-OHDA–lesioned drug-naive rats (PD/LD$^-$) ($n = 4$), 6-OHDA–lesioned LD-treated rats without dyskinesia (PD/DYS$^-$) ($n = 5$), and the sham/LD$^-$ ($p > 0.05$) groups. The inset on the right shows the significant interhemispheric difference between the lesioned (6-OHDA) and unlesioned hemispheres (represented by white-green blending color) within the PD/DYS$^+$ group. (B) Cortical Arc protein expression in the 5 groups: sham/LD$^-$ ($n = 4$), sham/LD$^+$ ($n = 4$), PD/LD$^-$ ($n = 4$), PD/DYS$^-$ ($n = 5$), and PD/DYS$^+$ ($n = 8$). In agreement with the gene expression, we found a significant difference between the PD/DYS$^+$ animals in comparison with the other groups ($p < 0.05$). We found a similar interhemispheric difference between the lesioned (6-OHDA) and unlesioned hemisphere (represented by white-green blending color) within the PD/DYS$^+$ group. *$p < 0.05$ each; data are expressed as mean ± standard error of the mean.

compared with other rats ($p < 0.001$). Because the state of phosphorylation of DARPP-32 represents a well-recognized sign of the dyskinetic state (Picconi et al., 2003), we performed a correlation analysis between the sleep-induced decrease of SWA (i.e., synaptic downscaling) and the content of striatal P-Thr34-DARPP-32 in all 5 groups of rats. In other words, we determined whether the P-Thr34-DARPP-32/DARPP-32 ratio parallels the impaired synaptic downscaling. Indeed, we found an inverse correlation between the increase in P-Thr34-DARPP-32 and the decrease in SWA during sleep ($p < 0.001$, $r = -0.70$, Fig. 8A).

We further extended our correlation analysis to the P-Thr34-DARPP-32/DARPP-32 ratio and the level of Arc protein measured at cortical level. In agreement with the results for the SWA, we also found a correlation between the P-Thr34-DARPP-32/DARPP-32 ratio and the content of Arc protein ($p < 0.001$, $r = 0.82$, Fig. 8B).

3.6. Measurement of corticostriatal-evoked potentials

In another set of experiments, we attempted to confirm the electrophysiological data described previously. For this purpose, we registered CS-EP in 3 animal groups (sham/LD$^-$, PD/DYS$^-$, and PD/DYS$^+$, $n = 10$ for each group) during the first and the last hours of the light period. In controls, we observed a potentiation of the CS-EP amplitude after high-frequency stimulation ($p = 0.06$ during early sleep and $p < 0.05$ during late sleep) followed by the physiological depotentiation after low-frequency stimulation that was

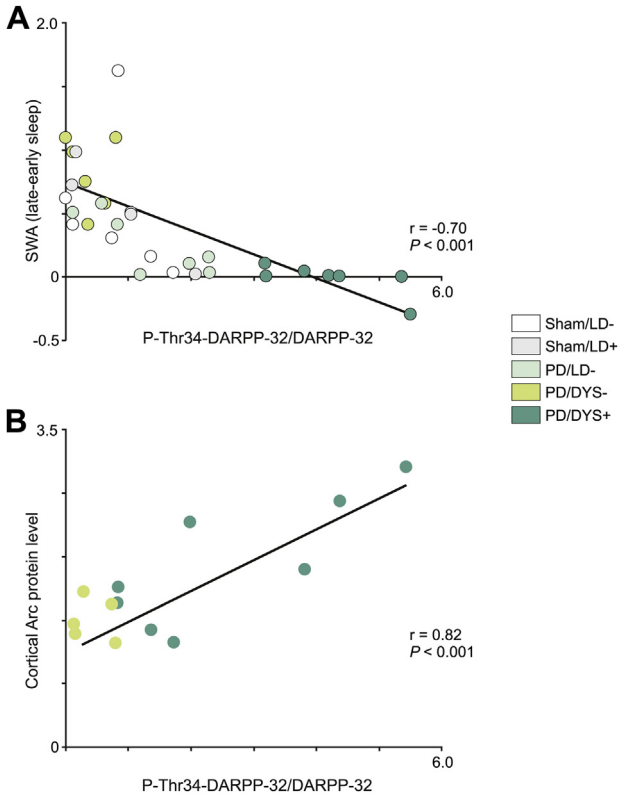

Fig. 8. Correlation between the striatal Thr34-phosphorylated-DARPP-32 (P-Thr34-DARPP-32)/DARPP-32 ratio and electrophysiological and molecular results. (A) Correlation between the difference in early-sleep and late-sleep slow-wave activity (SWA) and the P-Thr34-DARPP-32/DARPP-32 ratio in the 5 groups of rats: (i) sham-lesioned drug-naive rats (sham/levodopa [LD]$^-$) ($n = 6$), (ii) sham-lesioned LD-treated rats (sham/LD$^+$) ($n = 4$), (iii) 6-hydroxydopamine (6-OHDA)–lesioned drug-naive rats (PD/LD$^-$) ($n = 7$), (iv) 6-OHDA–lesioned LD-treated rats without dyskinesia (PD/DYS$^-$) ($n = 6$), and (v) 6-OHDA–lesioned LD-treated rats with dyskinesia (PD/DYS$^+$) ($n = 7$). The analysis showed a significant inverse correlation between the 2 parameters suggesting that the reduction of synaptic downscaling (as measured by the difference in SWA) is pronounced in animals with a higher P-Thr34-DARPP-32/DARPP-32 ratio, which is linked to the "dyskinetic state" of the animals ($p < 0.001$). (B) On the contrary, the P-Thr34-DARPP-32/DARPP-32 ratio is directly correlated to the cortical content of the Arc protein; in other words, those animals with a higher striatal P-Thr34-DARPP-32/DARPP-32 ratio have a higher content of Arc protein at the cortical level. Spearman correlation analysis: $p < 0.001$ and $^*p < 0.001$ 6-OHDA–lesioned LD-treated rats without dyskinesia (PD/DYS$^-$) versus PD/DYS$^+$. Data are expressed as mean ± standard error of the mean.

more pronounced during the last hour of the light period (both $p < 0.05$). In PD/DYS$^-$ rats, we found a significant potentiation and depotentiation only during the last hour of the light period (both $p < 0.05$). PD/DYS$^+$ rats showed a significant potentiation ($p < 0.05$), but without depotentiation ($p = 0.4$), only during the last hour of the light period (Fig. 9).

4. Discussion

Our data provide evidence that parkinsonian rats featuring dyskinetic movements have an altered mechanism of the physiological synaptic downscaling occurring during sleep. Dyskinetic movements are typical of the advanced stage of PD and can be functionally explained as intruding motor programs in selected neuronal responses operated by the basal ganglia leading to a perturbation of the desired motor planning by enduring signals (Calabresi et al., 2010). These assumptions arise from the finding that slices from LD-treated dyskinetic rats do not manifest the physiological depotentiation of corticostriatal-evoked responses to low-frequency stimulation of the corticostriatal synapse (Calabresi et al., 2000). The excess of LTP impairs the ability of the striatum to discriminate between relevant and irrelevant cortical inputs. We based our work on the assumption that the impairment of SH during the disease course reaches a critical point beyond which the lack of an adequate homeostatic renormalization of the cortico-cortical and corticostriatal synaptic strength leads to a runaway LTP. These effects alter the function of the corticobasal ganglia-thalamic loop because of imbalanced information storage (Calabresi et al., 2000; Huang et al., 2011; Picconi et al., 2003).

Herein, we observed that the degree of dopaminergic denervation does not have a significant effect on the percentage of time in wake, NREM sleep, and REM sleep. These results are in line with the previous findings showing only a transient alteration of the percentage of NREM and REM sleep within 1 week of dopaminergic lesion in rats treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Lima et al., 2007) but do not reflect the phenotype in PD patients showing a progressive reduction of the amount of NREM sleep during the disease course (Diederich et al., 2005). Besides, although the 5 animal groups were subjected to different treatments, there was no significant effect on the sleep macrostructure. We observed that dyskinetic animals show an impairment of NREM sleep–induced reduction of SWA. This electrophysiological parameter has been proposed as a marker of synaptic strength in cortical neurons and is physiologically reduced during NREM sleep by a homeostatic mechanism that prevents uncontrolled STDP (Hanlon et al., 2009; Tononi and Cirelli, 2014; Vyazovskiy et al., 2007). The physiological decay of SWA during sleep was significantly impaired in the lesioned hemisphere of dyskinetic animals; the similar pattern observed in the nonlesioned side, without reaching a significance, might be because of the interconnectivity between the 2 hemispheres.

To corroborate these data, we also analyzed CS-EPs of sham/LD$^-$, PD/DYS$^-$, and PD/DYS$^+$ rats. The obtained data showed a facilitating effect of sleep on corticostriatal synapses in sham/LD$^-$ rats. PD/DYS$^+$ animals were not able to depotentiate, which speaks in favor of uncontrolled LTP because of an impairment of SH as already suggested by the SWA analysis. PD/DYS$^-$ animals were able to depotentiate during late sleep and had a higher difference in SWA between early and late NREM sleep than the other groups, which may indicate the presence of a compensatory mechanism in this subpopulation.

Interestingly, an LD washout led to the reversal of impaired synaptic downscaling along with the clear reduction of involuntary movements in dyskinetic animals, possibly reflecting clinical reports describing a reduction of LID after a "drug holiday" in PD patients (Friedman, 1985; Koziorowski and Friedman, 2007). These results confirm the association between physiological NREM sleep underlying SH and the occurrence of LID. In agreement with the LD washout, we found a deterioration of dyskinesia along with a further impairment of synaptic downscaling after SD. Because it has been demonstrated that synaptic strength is built up during the day time and reduced at night time, it is tempting to speculate that this result reflects the clinical observation that dyskinesia is usually more pronounced during the afternoon or in the evening than in the morning. Besides, it can also be speculated that dyskinesia emerging during the course of disease may be associated with the increasingly altered sleep architecture observed in PD patients (Diederich et al., 2005).

SH can also be determined by measuring some molecules that can be implicated in this form of synaptic plasticity. One of these, Arc, plays a critical role in AMPAR endocytosis underlying STDP in F-actin formation that in turn underlies LTP consolidation (Bramham et al., 2008; Sgambato-Faure et al., 2005). In agreement with our

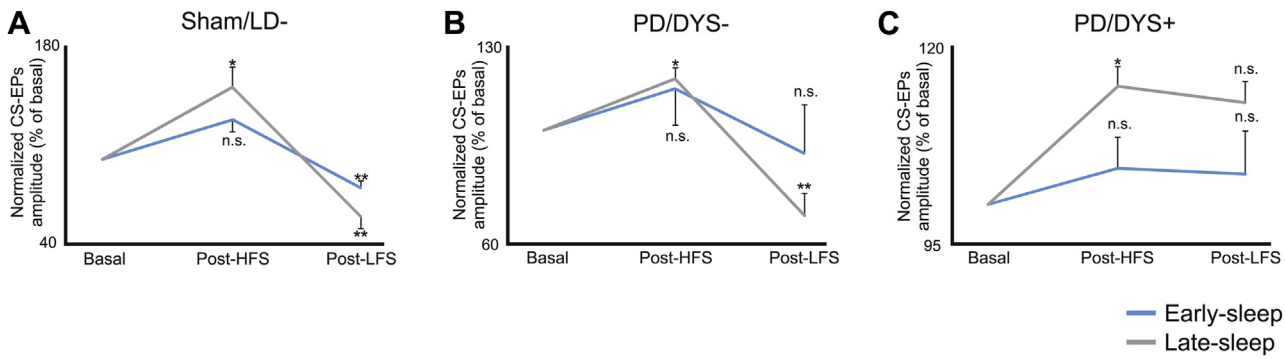

Fig. 9. Corticostriatal-evoked potential (CS-EP) recordings in 3 groups of animals during early and late sleep. (A–C) CS-EP in sham-lesioned drug-naive rats (sham/levodopa [LD]$^{-}$), 6-hydroxydopamine (6-OHDA)–lesioned LD-treated rats without dyskinesia (PD/DYS^{-}), and 6-OHDA–lesioned LD-treated rats with dyskinesia (PD/DYS^{+}) rats ($n = 10$ for each group) recorded during early (blue line) and late sleep (gray line). CS-EP amplitude was expressed as percentage of the basal value. $^{*}p < 0.05$ post-high frequency stimulation versus basal; post-low frequency stimulation versus post-high frequency stimulation; and ns, not significant. Data are expressed as mean ± standard error of the mean.

electrophysiological data, cortical Arc gene expression was enhanced in dyskinetic rats featuring impaired synaptic downscaling, whereas it was suppressed in PD/DYS^{-} rats. Accordingly, the overexpression of the Arc gene in dyskinetic animals and its suppression in animals treated with LD, but not showing dyskinesia, provide an additional indication that PD/DYS^{-} rats may make use of a compensatory mechanism. In agreement with the gene expression data, Arc protein was significantly augmented in PD/DYS^{+} rats but slightly increased in the PD/LD^{-} and PD/DYS^{-} in comparison with the sham groups.

The significant increase of Arc protein in dyskinetic animals at both the cortical and striatal levels corroborates the hypothesis of an uncontrolled LTP state in these animals. Indeed, the loss of LTP reversal, that is, depotentiation (Calabresi et al., 2000), is the electrophysiological counterpart of an augmented synaptic consolidation, in which Arc plays a crucial role (Bramham et al., 2008). To our knowledge, although an increased level of Arc protein has already been demonstrated within the striatum (Sgambato-Faure et al., 2005; Spinnewyn et al., 2011), and this is the first report showing an augmented Arc gene and protein expression in the cortex of dyskinetic animals. On the contrary, a clear correlation between Arc and homeostatic plasticity during sleep has been demonstrated (Cirelli and Tononi, 2000; Tononi and Cirelli, 2014). Moreover, we observed that the impairment of cortical SH, measured as persistent SWA during NREM sleep, in dyskinetic animals inversely correlates with the degree of phosphorylation of the Thr34 site in DARPP-32, a marker for changes at the postsynaptic level in these animals (Picconi et al., 2003). In accordance, we observed a significant direct correlation between the striatal P-Thr34-DARPP-32/DARPP ratio and cortical Arc protein levels. The impairment of SH with uncontrolled synaptic buildup suggested herein may be reflected by a recent report based on a voxel-based morphometry analysis of brain magnetic resonance imaging scans providing evidence of increased gray-matter volume (linked to synaptic connections as suggested by the authors) of the frontal cortex in dyskinetic PD patients (Cerasa et al., 2011). Of note, also in dystonia, another hyperkinetic movement disorder, an alteration of SH, has been proposed (Quartarone and Pisani, 2011).

Cortical activity reverberates to deep brain structures during physiological sleep and during anesthesia such as with urethane (Galati et al., 2009, 2010; Prosperetti et al., 2013; Steriade et al., 1993; Tseng et al., 2001). Thus, we can assume that during physiological sleep, homeostatic plasticity also affects the striatum and other basal ganglia structures exerting its synaptic downscaling function. In other words, SH probably involves the synapses not only at a cortical level but also at a striatal level. Our data seem to be in agreement with a failure of homeostatic plasticity in the striatum, as supported by the significant increase of the striatal Arc gene and protein. Over the short timescale of STDP, the impairment of adequate SH can lead to uncontrolled LTP not responding to depotentiation as observed in dyskinetic animals (Calabresi et al., 2010) and PD patients (Huang et al., 2011). This notion supports the intimate interplay between STDP and homeostatic plasticity sharing the same synaptic mechanisms that, however, work in different ways over short and long timescales of activity (Turrigiano and Nelson, 2004).

We are aware of 3 main limitations to our current work: (i) The data with respect to the expression of molecular markers of synaptic plasticity are intriguing but should be confirmed and extended in the future studies; (ii) although the CS-EPs are considered an adequate measure of local synaptic activity (Galiñanes et al., 2011), more conclusive data could be derived only from in vivo intracellular recordings that will be performed shortly; and (iii) the AIMs in rats cannot be directly compared with dyskinesia in PD patients, although the similarities are intriguing.

5. Conclusions

Our data provide the first evidence of an impaired SH in parkinsonian animals with dyskinesia, supporting the idea that sleep and its underlying mechanisms play a crucial role in the appearance of LID. During the course of PD, the occurrence of an inadequate downscaling of corticocortical and corticostriatal synaptic strength may alter the physiological function of basal ganglia. As a consequence, there may be an imbalanced information storage within the corticobasal ganglia-thalamic loop that produces persisting and widespread activity presenting as multiple interfering motor programs. From a clinical point of view, dyskinetic PD patients may benefit from compounds enhancing synaptic downscaling during NREM sleep.

Disclosure statement

The authors have no conflicts of interest to disclose.

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Appendix A. Supplementary data

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